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PII: S0308-8146(20)30056-X
DOI: https://doi.org/10.1016/j.foodchem.2020.126209
Reference: FOCH 126209

To appear in: *Food Chemistry*

Received Date: 24 September 2019
Revised Date: 11 November 2019
Accepted Date: 11 January 2020

Please cite this article as: Su, H., Tu, J., Zheng, M., Deng, K., Miao, S., Zeng, S., Zheng, B., Lu, X., Effects of Oligosaccharides on Particle Structure, Pasting and Thermal Properties of Wheat Starch Granules under Different Freezing Temperatures, *Food Chemistry* (2020), doi: https://doi.org/10.1016/j.foodchem.2020.126209

Effects of Oligosaccharides on Particle Structure, Pasting and Thermal Properties of Wheat Starch Granules under Different Freezing Temperatures

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Abstract: The effects of fructooligosaccharides (FOS), galactooligosaccharides (GOS), and xylooligosaccharides (XOS) on gelatinization, retrogradation, thermal properties and particle size of wheat starch at different freezing temperatures were studied. The results showed that the wheat starch porosity, particle size, crystallinity, gelatinization temperature, peak and breakdown viscosity increased with increasing freezing temperature. With the addition of 16% oligosaccharides to starch, the porosity, particle size, crystallinity, initial gelatinization temperature, peak value, breakdown and retrogradation viscosity of the starch granules significantly decreased in the order of XOS>GOS>FOS. However, the pasting temperature of the granules increased. The addition of oligosaccharides (especially XOS, which has the most significant effect in inhibiting starch retrogradation) can inhibit the formation of starch crystal structures to a certain extent, reduce the damage from ice crystals to starch granules and delay starch retrogradation. Therefore, functional oligosaccharides can be used as a potentially effective additive to increase freezing stability in frozen starch-based foods.

Keywords: wheat starch; oligosaccharides; prebiotic; freezing temperatures; retrogradation; pasting; thermal property

1. Introduction

Freezing treatment is often used in the preservation and drying process of starchy foods. Freezing dough is an important preservation technique in the storage of flour products. The dough is usually kept frozen, then thawed and baked before eating. However, with the increase of freezing time, the formation and recrystallization of ice crystals in dough leads to a decrease in yeast activity, weakening of the gluten structure and a decrease in the sensory properties of the products, making it difficult to restore all the original quality characteristics (Tao, Wang, Wu, Jin, & Xu, 2016c). The starch content in wheat flour is as high as 70-75% (A-type starch granule accounts for 70-80% of the total mass of starch, while B-type starch granule accounts for less than 10%). Starch is the largest part of dough solids (Colonna, Barry, Cloarec, Bornet, Gouilloud, & Galmiche, 1990), and it has an important impact on the quality of the final flour products by affecting water absorption in bread and thus the firmness of baked products (Gray & Bemiller, 2010). During freezing, water in starch granules is wrapped in the expanded channels, and a freezing treatment causes starch components to leach out by compressing the matrix of the starch granules, resulting in structural changes that can accelerate starch or starch food retrogradation (Han, Pei, Ali, Wu, Jin, & Xu, 2015).

The changes in starch molecular structure (ratio of amylose to amylopectin), starch solution concentration, ambient temperature and nonstarch components (salts, oligosaccharides, lipids and hydrocolloids) can affect the quality characteristics of frozen starch to a certain extent, and excessive intake of sweeteners, salts and lipids is not beneficial for human health (Jin, Fan, Ning, Pei, Jin, Lv, et al., 2013).

Oligosaccharides are soluble in water, and their sweetness is 0.3-0.6 times that of sucrose. Low sweetness makes them an ideal substitute for sucrose in foods for patients with diabetes, heart disease or thrombosis. Prebiotic additives can beneficially affect the health of the host. FAO/WHO defines prebiotics as an indigestible food ingredient that selectively stimulates the growth or activity of some microorganisms in the colon (e.g., *Lactobacillus* and *Bifidobacterium*). Some sources of probiotics include nondigestible carbohydrates, especially nondigestible oligosaccharides (mainly fructooligosaccharides (FOS, which are chains of fructofuranose residues linked by β (2→1)-linkages), galactooligosaccharides (GOS, which are complex oligosaccharides produced from lactose by galactosyl transfer reactions and linked mainly by β (1→4) and β (1 → 6)-linkages), and xylooligosaccharides (XOS, are mixtures of oligosaccharides formed by xylose residues linked through β (1→4)-linkages)), which meet all criteria for prebiotic classification (Pokusaeva, Fitzgerald, & Sinderen, 2011). Low-molecular-weight sugars have been widely used to improve the flavor and stability of starch foods. After adding oligosaccharides, they attach to the surface of starch granules or move into the interior of the starch granules in a starch solution to further interact, thereby increasing the peak viscosity and gelatinization temperature of starch and limiting the setback viscosity, swelling and amylose leaching of starch granules (Zhou, Bao, Bo, & Chen, 2017). Therefore, it is necessary to know the functional oligosaccharide-starch interactions under freezing conditions and the changes in starch granule quality to develop flour products with probiotic oligosaccharide components.

Frozen dough has been widely studied in the past few decades. Carbohydrate

applications include reducing the formation of ice crystals (Lu, Chen, Guo, Zheng, Rea, Su, et al., 2019). Studies on the changes in the starch granule properties in dough have mainly focused on the different freezing rates, times of freeze-thaw cycles, and particle sizes (Tao, Huang, Xie, Zou, Wang, Wu, et al., 2018; Tao, Wang, Wu, Jin, & Xu, 2016a, 2016c), but there have been few studies on the effects of probiotic oligosaccharides on the properties of starch granules in frozen dough at different freezing temperatures. The purpose of this study was to analyze the effects of functional oligosaccharides (FOS, GOS, and XOS) with probiotic functions on the properties, thermal and gelatinization properties of wheat starch granules at freezing storage temperatures, and the correlation between the properties of starch granules and the quality improvement of frozen dough after adding oligosaccharides was determined. It is very important to improve the quality and nutrition of the final thawed products.

2. Materials and methods

2.1 Materials

Wheat starch was obtained from Shanghai Lvyuan Starch Co., Ltd (Shanghai, China). Fructooligosaccharide (Meioligo-P®) was purchased from Meiji Seika Kaisha Ltd. (Tokyo, Japan) and contained 30% 1-kestose, 57% nystose, and 13% 1F-β-fructosylnystose. Xylooligosaccharide，XOS extracted from corncob (Xylooligo 95P，95％ XOS, Shandong Longlive Bio-Technology) with 84% XOS (43% DP=2, 30% DP=3, 10% DP=4, 17% DP≥5) and 13.5% other DP=1. Galactooligosaccharides, GOS (Nissin Sugar Mfg., Co., Ltd., Tokyo, Japan), comprised oligosaccharides with mostly DP=2–5. (3% DP=1, 21% DP=2, 52% DP=3, 19% DP=4, 5% DP>4). All other

chemicals and reagents were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were analytical grade unless stated otherwise.

2.2 Preparation of frozen starch

Accurately weighed 150±0.01 g of food grade wheat starch, added 3 g, 6 g, 12 g, 24 g, 48 g of FOS, XOS, GOS, and added 225 mL of deionized water by stirring for 30 min, the mixture was allowed to stand for 3 h to prepare a starch-oligosaccharide mixed solution having a concentration of 2%, 4%, 8%, 16% and 32% (w/w). The samples were placed in -20 ° C, -50 ° C and -80 ° C ultra-low temperature freezer (DW-HL388, Meiling Cryogenic Technology, China) for 10 weeks, and then taken out until the samples were completely thawed at room temperature. The upper pigmented layer was carefully removed after centrifugation at 5000 g for 10 min (Avanti J, Beckman, Fullerton, CA, USA). Repeat this process three times by magnetically stirred with adding deionized water and centrifuged to remove the oligosaccharides in starch. The starch was freeze-dried after rotary evaporation (Model fd-4c-80, Beijing Fuyikang Instrument Company, Beijing, China) to obtain powder. The dried starch samples were passed through a 100-mesh sieve for further study and conditioned at 25 ± 4 °C and 0% relative humidity for 10 days in a desiccator to get the similar moisture content.

2.3 True densities

The true density (T_d) was determined using a gas pycnometer (AccPyc II 1340 Pycnometer, Micromeritics Instrument Co., Norcross, GA， USA). 40 g wheat starch was compressed into a 50 mL cylindrical container for density measurement. The sample volume was measured by helium replacement (Daudt, Avena-Bustillos,

Williams, Wood, Külkamp-Guerreiro, Marczak, et al., 2016).

2.4 Bulk densities and porosity

5 g wheat starch was placed in 10 mL of measuring cylinder and the volume was noted. The bulk densities were calculated as the ratio of weight of wheat starch sample to occupied volume. Porosity (P_f) of starches was calculated as (formula (1)):

$$P_f = (1 - \frac{B_d}{T_d}) \times 100 \qquad (1)$$

Here, B_d is bulk density and T_d is true density. T_d were measured by the true densitometer.

2.5 Particle size distribution (PSD) analysis

The particle size distribution was determined using a laser diffraction analyzer (MasterSizer 3000, Malvern Instruments Ltd., Worcestershire, UK) equipped with a wet sample dispersion unit (Malvern Hydro MV, UK) and the Hydro EV accessory. The refractive index of optical properties used for water and starch was 1.36 (Lu, Chen, Zheng, Guo, Qi, Chen, et al., 2019). Starch powders were dispersed in deionized water before analysis and shaken sufficiently to ensure uniformity. The volume-based mean diameter (D[4,3]) were calculated as follows (formula (2)):

$$D[4,3] = \frac{\sum_i n_i d_i^4}{\sum_i n_i d_i^3} \qquad (2)$$

2.6 X-ray diffraction (XRD)

The crystalline structure of wheat starches was evaluated using an X-ray powder diffractometer (D8 Advance, Bruker, USA), operated at 40 kV and 30 mA. XRD patterns were acquired for a 2θ range of 5–40°, with a step size of 0.02° and a step rate of 0.5 s per step (Guo, Zeng, Lu, Zhou, Zheng, & Zheng, 2015). The relative

crystallinity (X_c, %) was calculated using the PeakFit software (Ver. 4.12) according to Eq. (3).

$$X_c = \frac{\Sigma_{i=1}^{n} A_{ci}}{A_t} \quad (3)$$

Where A_{ci} is the area under each crystalline peak with index i, and A_t is the total area of the diffraction pattern.

2.7 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the wheat starch was preparation by the KBr pellet method were measured using a VERTEX spectrometer (70v, Bruker Optik GmbH., Ettlingen, Germany). The spectra were acquired at wavelength between 400 and 4000 cm^{-1} with 4 cm^{-1} resolution with OPUS software (Version 7.0). All spectra recorded against an empty cell as the background were the averages of 64 scans with baseline corrected and normalized. The absorbance intensities of bands at about 1047, 1022 and 995 cm^{-1} were used to investigate the crystalline structures of the wheat starch.

2.8 Differential scanning calorimetry (DSC)

The thermal properties of wheat starch was analyzed using a differential scanning calorimeter (204 F1, Netzsch Group, Selb, Germany) equipped with a thermal analysis data station (Proteus® Software for Thermal Analysis ver. 6.1.0, Netzsch Group, Selb, Germany). Aliquots of water/starch mixtures (3 mg starch was added with 10 μL deionized water) were weighed accurately into 40 μL aluminum pans and detected after equilibrated overnight at room temperature (sealed with Tzero hermetic lid). An empty pan was used as the reference. The sample pans were heated from 30 to 100°C at 5°C/min under dynamic purge protective with nitrogen (20 mL/min) (Lu, Su, Guo, Tu,

Lei, Zeng, et al., 2019).

2.9 Viscosity measurement (RVA)

The pasting properties of native and frozen wheat starch starches under different freezing temperatures were measured with the Rapid Visco Analyzer (RVA-4500, Perten Instruments, Hägersten, Sweden). Starch (2 g based on 10% moisture content) was added to 20 mL of water placed into the aluminum RVA canister. Slurries underwent a controlled heating and cooling cycle (kept for 1 minute at 50℃, and then heat to 95℃ at 12℃/min rate and keep for 2.5 min, decrease to 50℃ at 12℃/min rate and keep for 2 min). The initial speed for mixing was 960 rpm for 10 s, followed by a 160 rpm paddle speed that was maintained for the rest of assay (Shao, Tsai, He, Chen, Wilson, & Lin, 2019). Pasting parameters were recorded using Thermocline software (Perten Instruments, Hägersten, Sweden).

2.10 Statistical analysis

All statistical performed was carried out using OriginPro 2017 (Origin Lab Corporation, Northampton, MA, USA), and the data are reported as the mean values ± standard deviation at least triplicate measurements. One way analysis of variance (ANOVA) followed by posthoc Duncan's test (p<0.05) was conducted to determine the significant differences among mean values using Data Processing System software (DPS, V9.05, Science Press, Beijing, China).

3. Results and discussion

3.1 True densities

True density refers to the actual mass of solid matter per unit volume under

absolute compactness, that is, the density after removing the internal pores and/or voids between particles, which reflects the helix tightness inside the starch granules. As the freezing temperature decreases, the true density of starch increases. It may be that the freezing treatment affects the internal structure of starch granules. After thawing, the water between the pores in starch granules and the amylose molecules in amorphous areas are discharged and then redistributed from the granules, resulting in an increase in pore size, shrinkage of the structure in the granules and a limited amount of water in the starch granules. That is, the volume of the starch granules increases, the intramolecular helix becomes tighter, and the true density increases (J. Szymońska, Krok, Komorowska-Czepirska, & bilas, 2003; Joanna Szymońska, Krok, & Tomasik, 2000).

Free water in starch granules changes into crystalline ice during the freezing process. Because ice crystals occupy more space than water in granules, the expansion force of ice crystals after freezing treatment enlarges the volume of starch granules, and pressure is generated, squeezing starch molecules inside the particles and leading to the formation of denser starch aggregates in samples with larger particle sizes. This change may be accompanied by the destruction of the original double helix by ice crystals in starch granules during freezing and the formation of an amylopectin double helix rejuvenate during thawing. Among these factors, the volumetric contraction of starch molecules brought about by retrogradation has a greater influence than the original double helix disruption of the particles.

Different concentrations of FOS, GOS and XOS were added to wheat starch under

different freezing temperatures. The true density of starch significantly decreased compared with that of frozen starch (p<0.05) (Table 1). The true density of starch treated with different oligosaccharides was lowest with XOS and higher for FOS and GOS, which were similar at some concentrations, possibly due to the interaction between sugar and water molecules (L. Wang, Xu, Fan, Wang, Wang, Zhang, et al., 2016). After adding sugar, the limited amount of free water in the starch granules reduces the amount of chilled water inside and reduces the shrinkage of the starch structure. The results show that the addition of oligosaccharides can reduce the pressure from ice crystal expansion on starch granules to some extent, thereby reducing the double helix effect among starch granules and the true density, and XOS are superior. The true density value of frozen starch no longer increased when the addition amount was 16%, so 16% was selected as the oligosaccharide addition concentration added to starch for subsequent research.

3.2 Bulk densities and porosity

The crystals at lower temperatures had lower values of bulk density (Zeng, Zhu, Chen, Gao, & Yu, 2016), the natural starch frozen at a higher temperature and the starch with added XOS showed lower bulk densities than the other samples (p<0.05). and the difference in the trend may be due to the difference in the number of crystals at different temperatures. Porosity (*Pf*) refers to the spatial parts of the granules that are not occupied by the granules. The particle size and shape of starch granules may lead to differences in density and porosity values (Deepika, Kumar, & Anima, 2013; Zhang, Zhu, He, Tan, & Kong, 2016). Free water in starch granules changes to crystalline ice

during freezing and causes damage to the starch granules. Due to the formation and gradual expansion of ice crystals during the freezing process, pressure on starch particles is produced, leading to swelling of the starch granules. In the process of thawing, the ice crystals melt, and water is released from the pores of the starch granules. The pressure generated by the ice crystals causes the starch granules to form a loose structure with pores after thawing. The internal and external molten ice crystals may result in pores on the surface of starch granules. The morphology and melting of ice crystals create a certain microscopic mechanical force on the starch granules, expanding the internal channel molecules of starch so that more soluble components are dissolved, water is redistributed, and more dents appear in the pores and starch granules (Yu, Zhang, Li, Yan, Gong, Liu, et al., 2015).

With increasing freezing temperature, the porosity of starch increases. It may be that higher freezing temperatures promote the formation of larger ice crystals and more damage to the starch granules, thus leading to an increase in starch porosity after thawing. The lower the temperature was, the smaller the ice crystals were. The slower the freezing rate and the larger the ice crystals, the larger the voids due to swelling were, resulting in an increase in porosity (Kono, Tobari, Araki, & Sagara, 2017).

When FOS, GOS and XOS were added to starch, the porosity of the starch significantly decreased (p<0.05) (Table 2). This change may be due to the addition of oligosaccharides decrease the frozen water content in the system and inhibit the formation of ice crystals in starch granules (Gunaratne, Ranaweera, & Corke, 2007). The results showed that oligosaccharides could inhibit the formation of pores by

reducing the grain damage caused by ice crystal expansion. The inhibition degree of different oligosaccharides on ice crystal formation after starch freezing is FOS>GOS>XOS, which shows that XOS is the most effective for increasing starch porosity. Due to its small molecular weight and low degree of polymerization, the molar mass of XOS is relatively large, the number of hydroxyl groups is relatively large, the hydration is strong, and the water content in starch is reduced, resulting in insufficient water to bind to starch molecules. The frozen water content is higher in starch, and the porosity is increased. In contrast, GOS and FOS have fewer hydroxyl groups due to their lower molar mass, resulting in fewer ice crystals and lower porosity than XOS.

3.3 Particle size distribution (PSD) analysis

Wheat starch can be divided into large type-A (10-40 μm) and small type-B (1-10 μm) starch according to its particle size. The wheat starch used in the experiment is mainly type-A starch and contains a small amount of type-B starch. Compared with type-A starch, type-B starch has less amylose, smaller volume and larger specific surface area but higher protein and lipid contents. B-type starch is easier to break during the milling process and has high water absorption, which affects the kneading and baking properties of dough (Tao, Wang, Wu, Jin, & Xu, 2016c).

The grain size of unfrozen wheat starch D[4,3] is 16.28 μm (Table 2). With the increase in freezing temperature, the D[4,3] of wheat starch significantly increases after freezing (p<0.05), which indicated that the starch granules expanded after freezing treatment at lower temperatures. This result may be due to the change of free water in starch granules into crystalline ice during the freezing process, and the crystalline ice

swelling exerts an outward pressure to increase the volume of the starch granules, which results in an increase in the particle size.

When FOS, GOS and XOS were added to starch, the particle size of starch significantly decreased. Oligosaccharides reduce the freezing point of a solution due to hydration, resulting in smaller ice core formation. The formation of smaller ice crystal samples may also lead to a larger particle size range of wheat starch. In addition, this difference may be related to the interactions of components (mainly protein and fat) on the surface of the frozen granules. There are more starch granule-related proteins and bound lipids in wheat starch, which tend to restrict the interaction between starch and oligosaccharides. The hydration ability of oligosaccharides is related to the number of hydroxyl groups in monosaccharide molecules, which interact with water molecules to form hydrogen bonds and enhance the hydration ability of sugars, and the XOS and GOS molecule hydroxyl number is more than that of and FOS (due to the lower relative molecular weight), so their hydration ability is XOS>GOS>FOS. Therefore, the interaction force between FOS-starch may be stronger than that for XOS and GOS, so the protein/lipid-surrounded particles are relatively weak and the particle size is relatively low (Pourmohammadi, Abedi, Hashemi, & Torri, 2018), which is consistent with the porosity results.

3.4 X-ray diffraction

Starch is a natural polycrystalline polymer. Its particles can be divided into amorphous, subcrystalline and crystalline regions according to structural differences. The X-ray diffraction pattern of the crystalline structure shows peak diffraction

characteristics, while amorphous and subcrystalline structures show dispersion peak diffraction characteristics. The formation of dispersion peaks in the amorphous region is due to the existence of short-range ordered and long-range disordered molecules in this region, while the dispersion peaks formed by subcrystallites are due to small crystal grains or incomplete crystallization. Crystal structure, unit cell size, and regular and ordered double helical structure changes in the regeneration process can be detected by XRD technology, reflecting the type, degree and three-dimensional order of starch crystallization (S. Wang, Li, Copeland, Niu, & Wang, 2015).

The X-ray diffraction patterns of wheat starch at all freezing temperatures have strong diffraction peaks at 15.4°, 17.0°, 18°, 20.0° and 23.0° (showing a typical A-type diffraction peak) (Fig. 1), and there are double peaks at 17.0° and 18.0°. The diffraction peak at 20.0° indicates that the amylose-lipid crystalline complexes are present in the particles (C. Liu, Wang, Chang, & Wang, 2015), which indicates that the freezing temperature has no significant effect on the starch structure and does not change the crystal type (Y. Liu, Gao, Wu, Gou, Jing, Zhao, et al., 2019). Wheat starch is a semicrystalline structure, and its crystalline region is mainly composed of a double helix structure of amylopectin inside the granules. During the starch freezing-thawing process, with the loss of water, the distance between starch molecular chains is shortened, and the molecular chains are rebonded by hydrogen bonds, gradually forming a preliminary crystal structure with small grain linearity and incomplete crystal shape. However, this structure does not show the peak diffraction characteristics of microcrystals, but only the broadened amorphous dispersion diffraction characteristics,

which is the submicrocrystalline structure.

The increase in the crystallinity of starch granules at lower temperature may be due to the double helix rearrangement of amylopectin in the crystalline zone under low-temperature freezing conditions after thawing, which transforms the subcrystalline crystals into the crystalline zone. Then, a more orderly and stable double helix structure is formed, which improves the proportion of the crystallization region and crystal integrity. The lower crystallinity at higher temperature may be due to the destruction of the original branched double helix structure of amylopectin starch during freezing being greater than the starch retrogradation effect during thawing, resulting in a reduction in the order of starch crystallization. The crystallization tendency is different from that reported by Tao et al. (Tao, Wang, Wu, Jin, & Xu, 2016b), probably due to differences in the starch water holding capacity due to different freezing endpoint temperatures or smaller ice crystal size at freezing temperatures of -30 ℃ or lower. There was not enough to damage the order of the original crystallized area.

Compared with the native starch, all the diffraction peaks of frozen starch after adding FOS, GOS and XOS did not change, and they still indicated type A crystal structure. However, the peak intensity decreases and the crystallinity of the starch decreases significantly (p<0.05) (Table 2). The inhibition of oligosaccharides on starch crystallinity was XOS>GOS>FOS, indicating that the addition of low-molecular-weight sugars can decrease the degree of starch crystallization and crystal lattice ordering, which may be due to the interaction between oligosaccharides and water molecules. The hydrophilic action of sugar competes for free water between starch

molecules, which leads to reduced contact between starch and free water, inhibiting the reaggregation of starch molecules to form a double helix and reducing its crystallinity due to the destruction of ice crystals or promoting the unwinding of the original amylopectin molecule. With the addition of sugar molecules, the ordered double-helical starch molecules in the crystallization zone are gradually transformed into relatively disordered subcrystals and amorphous regions by the destruction of large ice crystals (the crystalline region of FWS-XOS and FWS-FOS transformed into an amorphous region; the FWS-GOS region transformed into a subcrystalline region).

3.5 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR) can detect the degree of starch retrogradation. The characteristic peaks in the 800-1300 cm^{-1} wavelength range are mainly caused by C-O and C-C stretching vibrations, which can reflect the conformation and hydration degree of a starch polymer. The infrared spectra of this region are mainly characterized by three modes. The characteristic peaks of maximum absorbance are 1047, 1022 and 995 cm^{-1}. The absorption bands at 1047 cm^{-1} and 995 cm^{-1} are related to the starch crystallinity and the short-range order of the double helix, respectively. Absorption bands at 1022 cm^{-1} are mainly related to the starch amorphous structure (S. Wang, Wang, Zhang, Li, Yu, & Wang, 2015). Absorption peak intensity ratios of 1047/1022 cm^{-1} ($R_{1047/1022}$) and 995/1022 cm^{-1} ($R_{995/1022}$) can be used to represent the starch crystallinity and molecular-order degree, respectively (Chen, Li, Mao, Huang, Wang, Qu, et al., 2017).

With the decrease in freezing temperature, wheat starch $R_{1047/1022}$ showed an

upward trend (Table 2). This result may be because amylose molecules in wheat starch before freezing hinder the migration of amylopectin and restrain the structural reorganization of amylopectin. Amylose dissolves after freezing, causing amylopectin rearrangement, the formation of a more ordered double helix structure and increased crystallization of the starch crystal region (Yu, Ma, & Sun, 2009). This is in accordance with the XRD test results. Among them, the ice crystals formed at freezing temperatures of -20 and -50 °C have a great degree of disorder in the starch crystal order, which leads to a lower $R_{1047/1022}$ than natural starch. $R_{995/1022}$ reflects the order of the molecular helix structure formed by wheat starch freezing. The absorption degree increases with decreasing temperatures, indicating that a lower freezing temperature is conducive to the formation of a starch double helix, which may be due to the high moisture content retained in starch molecules.

The $R_{1047/1022}$ value decreased significantly after adding FOS, GOS and XOS to wheat starch at different freezing temperatures (p<0.05), indicating that the addition of low-molecular-weight sugars can promote the disorder of the original amylopectin crystallization regions during freezing but inhibit the retrogradation crystallization of amylopectin during thawing. The crystallization inhibition effect is XOS>GOS>FOS, which may be related to the relative molecular weight; i.e., XOS is lower and the relative molecular weight of GOS is higher. In addition, related to the molecular weight and monosaccharide composition of the oligosaccharides, as a kind of five-carbon sugar, XOS have a relatively low molecular weight compared with that of six-carbon sugars; thus, the number of molecules in solution is higher at the same mass concentration, so

that the number of hydroxyl groups with which hydration can occur is greater than that of six-carbon sugars. Therefore, the overall interaction of XOS in a starch system is stronger.

The number of double helices is FOS>GOS>XOS, which is different from the trend of porosity and particle size. The addition of oligosaccharides has a strong ability to destroy the double helix, indicating that it is not only the destruction from frozen ice crystals. This may also be because oligosaccharides are mainly involved in promoting unwinding after crosslinking interaction with amylopectin in the crystalline region of the original starch granules. Second, large ice crystals are formed by the poor hydration of oligosaccharides, which may significantly destroy the double helix of the starch granule and the degree of order of the damaged crystal region. Finally, the interaction between oligosaccharides and water molecules inhibits the binding of starch and water and the reaggregation of starch molecules, thereby reducing retrograde crystallinity.

The addition of oligosaccharides showed similar trends in the $R_{995/1022}$, $R_{1047/1022}$ and XRD results, indicating that the increase and decrease in the number of double helices before and after freezing determined the short-range and long-range ordered structure of the starch crystallization regions, indicating that oligosaccharides are important influences on starch double helices.

3.6 Differential scanning calorimetry

Gelatinization is a swelling-driven process in which the expansion of the amorphous region destroys the crystalline domains because the gelation process generates stress (Sopade, Halley, & Junming, 2004). The initial gelatinization

temperature (T_0), peak temperature (T_p), termination temperature (T_c) and gelatinization enthalpy (ΔH) of wheat starch increased with the decrease in the freezing temperature (Table 3). This may be due to the separation of amylose molecules in the amorphous region of wheat starch after freezing, which results in the reaggregation of amylopectin and the formation of a more orderly and stable double helix structure, thus increasing the crystallinity and stability of starch crystals. The energy required for melting the double helix structure of starch chains during heating is increased. The initial gelatinization temperature and gelatinization enthalpy of starch are increased, indicating that more energy is needed to destroy the starch granule structure. However, the ΔH values at -20 and -50 °C are lower than that of natural starch, which may be due to the ice crystals destroying the order of the crystalline regions and the double helix and causing a low level of retrogradation. The ΔH at -80 °C is relatively high because during the cooling of starch paste, a stable three-dimensional structure is again formed by hydrogen bonding, and the energy required to destroy the double helix generated by retrogradation is higher than the energy required for the amylopectin double helix of the original starch granule (Luo, Li, Xu, Ren, Li, Li, et al., 2017).

Compared to frozen starch, the addition of FOS, GOS and XOS significantly decreased the T_0, T_p, T_c and gelatinization temperature (p<0.05), indicating that oligosaccharides accelerated the initial gelatinization temperature (from the results of T_0) of microcrystals in granules and the degree of granule expansion during gelation (Lai, Shiau, & Wang, 2012). On the one hand, the interaction between oligosaccharides and water molecules may decrease the water activity, increase the interaction between

starch molecules, as well as sugar-starch molecules, and further improve the plasticization of starch (L. Wang, et al., 2016).

On the other hand, as a polysaccharide with low molecular weight, oligosaccharides are easy to insert into the interior of starch molecules. The amylopectin double helix in starch granules unwinding promotes the dissolution of amylose in starch molecules. By excluding steric hindrance, the binding of amylopectin is unrestricted to form a double helix, which promotes the expansion of starch particles. Starch retrogradation is inhibited after sugar addition, and less energy needs to be absorbed to complete gelatinization, resulting in ΔH being consistent with the change in the trend of crystallinity.

3.7 Viscosity measurement

Starch granules with water absorption properties expand after heating, and the structure changes irreversibly from ordered to disordered. Amylose molecules in the amorphous region in the granules dissolve out when heated, but the crystalline region remains stable. With increasing temperature, heat energy leads to swelling of crystallized starch, and a large number of water molecules enter the starch granules. After the granules are broken, a loose gel-like dispersed phase is formed, and gelatinization occurs. The dissolved amylose and amylopectin interact to form a three-dimensional network structure, and the viscosity of the dispersion system is maximized. After gelatinization, the cluster structure of the starch dissociates, and the cross-polarization phenomenon disappears, forming a translucent high-viscosity colloid. In the heating process, the temperature at which the viscosity of starch milk begins to rise

rapidly is named the pasting temperature. The gelatinization temperature of starch increases with the decrease in the freezing temperature (Table 4 and Fig. 2), which may be due to the increase in the crystallinity of starch granules at lower freezing temperatures and the increase in energy required to destroy the stable structure during gelatinization. After adding FOS, GOS, and XOS, the paste temperature of wheat starch with high-molecular-weight or six-carbon oligosaccharides increased further. This result may be due to the penetration of low-molecular-weight sugars into the starch granules and the starch granules forming a complex by crosslinking (sugar bridge) between the starch chains in the amorphous regions of the starch granules, which stabilizes these regions (Zhou, Bao, Bo, & Chen, 2017). The results indicate that FOS may have a stronger interaction with starch and result in the highest paste temperature.

Peak viscosity refers to the viscosity of starch granules when fully gelatinized, which may be related to the swelling ability of starch granules (Zavareze & Dias, 2011). Amylopectin is an important factor affecting the expansion of starch granules, while amylose and lipids inhibit the expansion of starch granules and maintain the integrity of expanded starch granules. The higher the freezing temperature is, the more amylose will be dissolved from starch molecules, and the proportion of amylopectin will increase, which increases the peak viscosity of starch. The addition of FOS, GOS, XOS may add interaction between oligosaccharides and the amylopectin, resulting in a decrease in the starch swelling capacity and peak viscosity. The increase in the paste temperature and the decrease in the peak viscosity indicates that oligosaccharides inhibit and expand starch granules and delay the formation of a viscous paste during

heating. Considering that oligosaccharides may have a higher ability to interact with water than starch, the addition can reduce the amount of available water for starch gelatinization (Angioloni & Collar, 2009).

The breakdown viscosity represents the shear resistance abilities of granules during heating. A higher breakdown viscosity indicates that the grain crack or starch is less likely to resist shear force and is susceptible to destruction during heating. The increase in the breakdown viscosity of starch after low-temperature freezing may be due to the dissolution of amylose during freezing, which leads to a decrease in stability as well as a decrease in the stability of the starch paste during heating (Yana, Evelyn, Siti, Herlina, Edy, & Dian, 2019). Oligosaccharides can interact with starch granules, inhibit starch-water binding and starch-starch reaggregation, and improve freezing stability. That is, the addition of FOS, GOS and XOS can inhibit starch granule destruction and starch molecular leaching after starch is subjected to thermal stress and shear stress, thus reducing the breakdown viscosity. The setback viscosity represents the difference between the valley viscosity and final viscosity, reflecting the secondary increase in the viscosity in the cooling stage. This change is mainly due to the rearrangement between amylose and amylopectin. After freezing at low temperature, the amylose in wheat starch dissolves out, and amylopectin rearranges to form a more ordered double helix structure, which leads to an increase in the retrogradation viscosity of starch, indicating that frozen starch has a higher retrogradation tendency or a lower retrogradation resistance. This result is consistent with the crystallinity and ΔH (Charles, Cato, Huang, Chang, Ciou, Chang, et al., 2016). Adding FOS, GOS and XOS to starch

for freezing treatment resulted in a decrease in the starch retrogradation viscosity. This result may be due to the interaction between oligosaccharides and water molecules, which inhibit the binding of starch and water and the reaggregation of starch molecules, thus delaying the retrogradation of starch and reducing the retrogradation viscosity.

Along with the above findings, it is proposed that the double helix produced by the re-crystallization of the starch-retrogradation system could be defined by true thickness, essentially preventing the overlay impact of conflict between the initial double helix breakage of the starch granules and the re-formation of the double helix in the IR, XRD, DSC and RVA experiments. When the concentration of oligosaccharides in wheat starch reaches 16 per cent, indicating that it has the best inhibitory effect on starch reduction, because true density does not increase with concentration growth. Effects of frozen ice crystals on the structural properties of starch granules may be inhibited by oligosaccharides, which may be due to the interaction between oligosaccharide-free water and oligosaccharide starch. This has the capacity of inhibiting the combination of starch and water and the re-aggregation of starch molecules to improve the phenomenon of starch retrogradation and forms a more ordered crystal structure. As a result, the temperature of starch gelatinization and gelatinization of the enthalpy increase. The free water content of starch can play a key role in its crystallinity. When freezing temperatures are higher, free water from starch molecules is more likely to precipitate and form larger ice crystals. The ice crystals formed by precipitated free water from the original starch particles will make the amylopectin double helix, and the degree of order in the crystalline region will be

destroyed (including the destruction of short-range and long-range orderly structures). Retrogradation may be exacerbated by the water content, which has a greater effect than steric obstruction due to amylose precipitation from starch granules, resulting in re-hyperhelix caused by the regenerative amylopectin after thawing. At the same time, under the condition of ultra-low temperature, the free water lost from the starch granules is lower than at high temperature, which results in a higher water holding capacity of the starch. Due to the high water content of starch molecules, amylopectin or amylose retrograde is stimulated during unfreezing (caused by hydrogen bonding in the double helix of starch). The increase and decrease in total starch crystallinity are closely related to the destruction of ice crystals during freezing and the reduction in starch during thawing. In the case of the same additional mass, functional oligosaccharides with a lower average relative molecular weight have more hydration due to the higher number of molecules in the system, and the water in the starch granules can reduce and inhibit retrogradation. The formation of ice crystals and the interaction between oligosaccharides and starch molecules can reduce the starch granules' crystallization area, all of which contribute to the starch structure's stability.

4. Conclusions

Gelatinization, retrogradation, thermal properties and particle size changes of starch granules with wheat starch or starch-oligosaccharide (FOS, XOS, GOS) in this study, mixtures at different freezing temperatures were studied. The results show that the lower freezing temperature has a positive correlation with the decrease in starch. While oligosaccharides may inhibit the damage to the starch structure caused by

freezing and starch retrogradation. With the addition of oligosaccharides, the true density, porosity, retrogradation and swelling of starch granules decreased due to ice crystal expansion caused by freezing, and XOS had the most significant inhibitory effect on starch retrogradation. Oligosaccharides can therefore effectively increase the freezing stability of wheat starch and have the potential to improve the aging and regeneration of wheat starch food, depending on the type and concentration of oligosaccharides.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements: This study was financially supported by the Research Fund for the National Natural Science Foundation of China (No. 31601473), the Science and Technology Major Project of Fujian Province of China (No. 2018NZ0003-1), the Natural Science Foundation of Fujian Province of China (No. 2018J01697), the FAFU Funds for Distinguished Young Scientists (xjq201811), the Research Fund for the China-Ireland International Cooperation Centre for Food Material Science and Structure Design (No. KXGH17001), Projects for Scientific and Technological Development of Fujian Agriculture and Forestry University (CXZX2018069), the Program for Innovative Research Team in Science and Technology in Fujian Province University (2012-03), and the Scientific and Technological Innovation Team Support Plan of Fujian Agriculture and Forestry University (cxtd12009).

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H.S, prepared this paper, the experiments as well as partial experiments to analyze the structure; J.J.T. conducted the RVA and DSC experiments; M.J.Z and K.B.D conducted tests to analyze the IR and XRD; S.M. performed densities and particle size experiments; S.X.Z. and B.D.Z. analyzed the statistical analysis; and the project was under supervision of X.L. and S.X.Z. All authors discussed and commented on the manuscript.

Figure captions

Figure 1. XRD patterns of native wheat starch and starch-oligosaccharides at different freezing temperatures. (A) -20℃; (B)-50℃; (C) -80℃. (NWS: native wheat starch; FWS: freezing-treated wheat starch; FOS: freezing-treated wheat starch with fructooligosaccharides; XOS: freezing-treated wheat starch with xylooligosaccharides; GOS: freezing-treated wheat starch with galactooligosaccharides.)

Figure 2. RVA curves of native wheat starch and starch-oligosaccharides at different freezing temperatures. (D) -20℃; (E)-50℃; (F) -80℃. (NWS: native wheat starch; FWS: freezing-treated wheat starch; FOS: freezing-treated wheat starch with

fructooligosaccharides; XOS: freezing-treated wheat starch with xylooligosaccharides; GOS: freezing-treated wheat starch with galactooligosaccharides.)

Fig.1

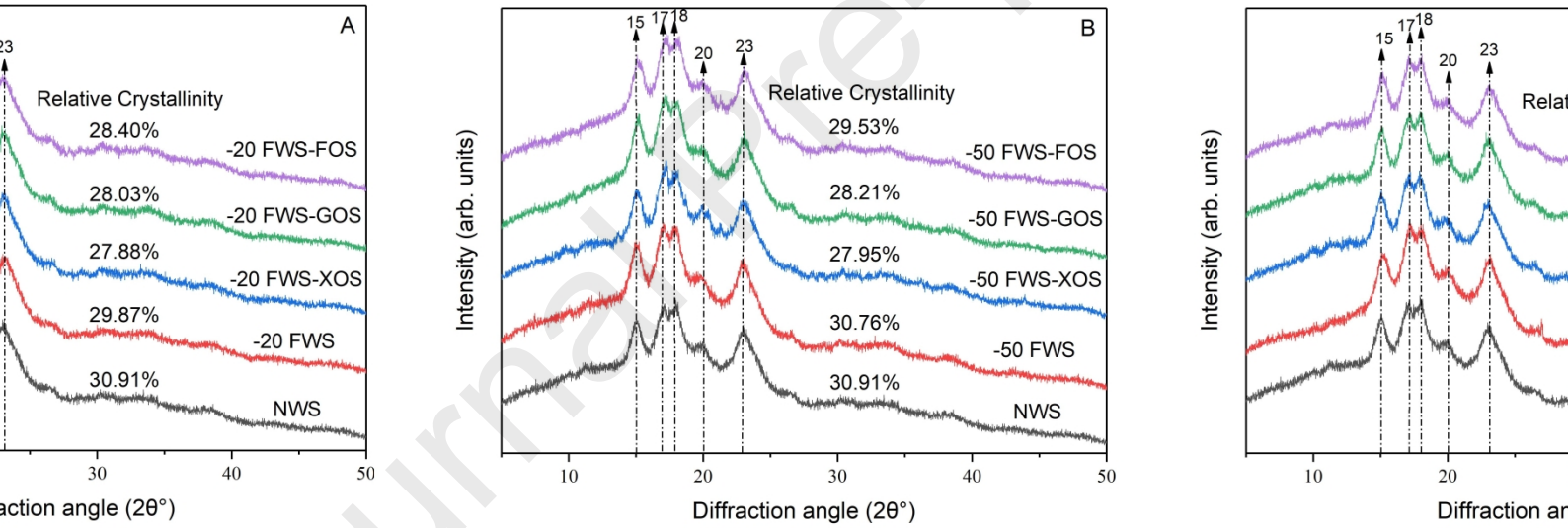

Fig.2

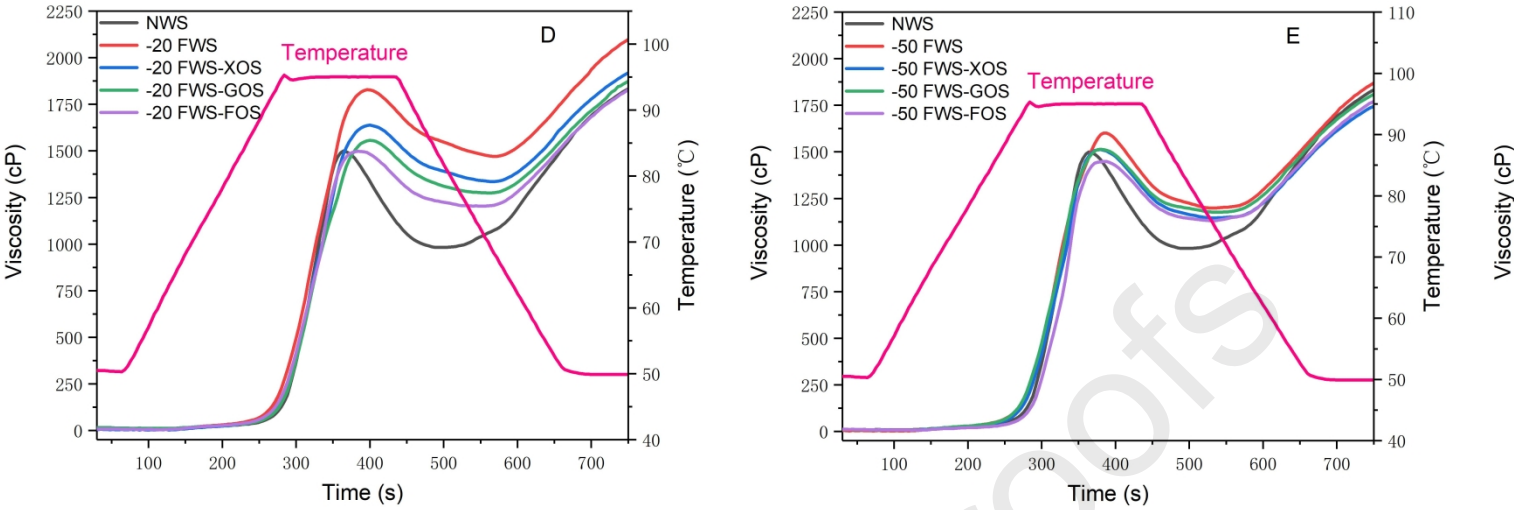

Table 1 True density of native wheat starch and starch-oligosaccharides at different freezing temperatures. (NWS: native wheat starch; FWS: freezing-treated wheat starch; FOS: freezing-treated wheat starch with fructooligosaccharides; XOS: freezing-treated wheat starch with xylooligosaccharides; GOS: freezing-treated wheat starch with galactooligosaccharides.)

	Starch concentration	Temperature	NWS	FWS	FWS-XOS	FWS-GOS	FWS-FOS
True density(g/cm^3)	2%	-20	1.471±0.002^a	1.517±0.004^b	1.477±0.002^c	1.488±0.001^d	1.484±0.002^d
		-50		1.515±0.003^b	1.453±0.002^c	1.485±0.002^d	1.494±0.002^e
		-80		1.528±0.001^b	1.495±0.002^c	1.506±0.000^d	1.494±0.002^c
	4%	-20		1.517±0.004^b	1.469±0.002^a	1.487±0.002^c	1.482±0.003^d
		-50		1.515±0.003^b	1.452±0.002^c	1.482±0.003^d	1.490±0.002^e
		-80		1.528±0.001^b	1.476±0.003^c	1.502±0.003^d	1.494±0.001^e

		-20	1.517±0.004[b]	1.467±0.003[a]	1.486±0.001[c]	1.477±0.003[d]
	8%	-50	1.515±0.003[b]	1.452±0.002[c]	1.479±0.002[d]	1.489±0.003[e]
		-80	1.528±0.001[b]	1.476±0.002[c]	1.502±0.001[d]	1.492±0.002[e]
		-20	1.517±0.004[b]	1.436±0.002[c]	1.449±0.001[d]	1.453±0.003[e]
	16%	-50	1.515±0.003[b]	1.444±0.002[c]	1.478±0.001[d]	1.483±0.004[e]
		-80	1.528±0.001[b]	1.474±0.001[a]	1.488±0.001[c]	1.491±0.003[c]
		-20	1.517±0.004[b]	1.457±0.001[c]	1.494±0.003[d]	1.469±0.002[a]
	32%	-50	1.515±0.003[b]	1.448±0.002[c]	1.495±0.003[d]	1.485±0.002[e]
		-80	1.528±0.001[b]	1.476±0.001[c]	1.492±0.002[d]	1.491±0.002[d]

Mean of three measurements ± standard deviation.
Values for the same processing conditions and different additive groups with different letters are significantly different (p<0.05).

Table 2 Bulk densities, porosity, particle size, XRD patterns and selected FTIR peak ratios characteristics of native wheat starch and starch-oligosaccharides at different freezing temperatures. (NWS: native wheat starch; FWS: freezing-treated wheat starch; FOS: freezing-treated wheat starch with fructooligosaccharides; XOS: freezing-treated wheat starch with xylooligosaccharides; GOS: freezing-treated wheat starch with galactooligosaccharides.)

	Index	Temperature	NWS	FWS	FWS-XOS	FWS-GOS	FWS-FOS
Bulk densities and Porosity	Bulk densities(g/cm^3)	-20	0.99±0.00[a]	0.79±0.00[b]	0.76±0.00[c]	0.78±0.00[d]	0.79±0.00[e]
		-50		0.81±0.00[b]	0.80±0.00[c]	0.82±0.00[d]	0.84±0.00[e]
		-80		0.87±0.00[b]	0.91±0.00[c]	0.93±0.00[d]	0.94±0.00[e]
	Porosity(%)	-20	32.32±0.04[a]	48.11±0.08[b]	47.31±0.13[c]	46.24±0.09[d]	45.53±0.04[e]
		-50		46.61±0.12[b]	44.30±0.01[c]	44.21±0.07[c]	43.41±0.06[d]
		-80		43.25±0.05[b]	38.05±0.09[c]	37.80±0.05[d]	36.88±0.05[e]

PSD	$D_{[4,3]}$(μm)	-20	16.28±0.03[a]	23.91±0.02[b]	19.02±0.02[c]	19.00±0.00[c]	18.92±0.00[d]
		-50		21.76±0.02[b]	18.71±0.03[c]	18.62±0.02[d]	18.44±0.04[e]
		-80		19.01±0.01[b]	18.33±0.03[c]	18.15±0.05[d]	17.91±0.05[e]
XRD	Crystal pattern		A-type	A-type	A-type	A-type	A-type
	Proportion of the micro-crystalline region(%)	-20	30.91±0.05[a]	29.87±0.08[b]	27.88±0.07[c]	28.03±0.06[d]	28.40±0.04[e]
		-50		30.76±0.10[b]	27.95±0.08[c]	28.21±0.08[d]	29.53±0.09[e]
		-80		31.88±0.05[b]	28.71±0.11[c]	29.01±0.05[d]	30.35±0.10[e]
	Proportion of the sub-crystalline region(%)	-20	28.25±0.20[a]	30.92±0.17[a]	27.31±0.12[b]	32.84±0.17[c]	31.63±0.25[d]
		-50		36.59±0.16[b]	27.93±0.20[c]	32.04±0.15[d]	28.65±0.21[e]
		-80		29.23±0.15[b]	33.79±0.07[c]	35.58±0.17[d]	28.60±0.17[e]
	Proportion of the none-crystalline region (%)	-20	40.85±0.21[a]	39.21±0.11[b]	44.81±0.14[c]	39.13±0.06[b]	39.97±0.12[d]
		-50		32.65±0.14[b]	44.12±0.15[c]	39.74±0.19[d]	41.82±0.10[e]
		-80		38.90±0.09[b]	37.49±0.20[c]	35.42±0.16[d]	41.05±0.08[e]
FTIR	$R_{1047/1022}$	-20	0.979±0.002[a]	0.969±0.002[b]	0.937±0.003[c]	0.948±0.005[d]	0.952±0.005[d]
		-50		0.977±0.001[a]	0.948±0.002[b]	0.959±0.003[c]	0.962±0.004[c]
		-80		0.984±0.003[b]	0.952±0.004[c]	0.965±0.002[d]	0.973±0.002[e]
	$R_{995/1022}$	-20	0.962±0.003[a]	0.944±0.001[b]	0.919±0.004[c]	0.928±0.001[d]	0.933±0.003[e]
		-50		0.958±0.004[a]	0.934±0.004[b]	0.943±0.005[c]	0.950±0.003[c]
		-80		0.980±0.005[b]	0.943±0.005[c]	0.957±0.005[d]	0.968±0.002[e]

Mean of three measurements ± standard deviation.
Values for the same processing conditions and different additive groups with different letters are significantly different (p<0.05).

Table 3 Gelatinization parameters of native wheat starch and starch-oligosaccharides at different freezing temperatures. (NWS: native wheat starch; FWS: freezing-treated wheat starch; FOS: freezing-treated wheat starch with fructooligosaccharides; XOS: freezing-treated wheat starch with xylooligosaccharides; GOS: freezing-treated wheat starch with galactooligosaccharides.)

Index	Temperature	NDS	FWS	FWS-XOS	FWS-GOS	FWS-FOS
T_o (°C)	-20	60.43±0.18[a]	63.75±0.15[b]	59.84±0.21[c]	59.72±0.22[c]	59.19±0.18[d]
	-50		63.31±0.22[b]	59.36±0.24[c]	62.83±0.09[d]	63.30±0.14[b]
	-80		66.73±0.14[b]	63.80±0.06[c]	63.79±0.14[c]	64.59±0.06[d]
T_p (°C)	-20	65.02±0.15[a]	68.34±0.04[b]	63.72±0.15[c]	64.08±0.13[d]	63.30±0.17[c]
	-50		68.27±0.23[b]	63.45±0.08[c]	66.22±0.12[d]	67.82±0.18[e]
	-80		68.38±0.25[b]	67.72±0.22[c]	67.71±0.11[d]	68.64±0.23[b]
T_c (°C)	-20	69.69±0.19[a]	72.19±0.15[b]	65.07±0.14[c]	65.17±0.08[c]	67.63±0.24[d]
	-50		73.18±0.25[b]	67.95±0.18[c]	70.04±0.13[d]	72.38±0.19[e]
	-80		71.84±0.11[b]	71.40±0.18[c]	73.62±0.15[d]	72.93±0.18[e]
ΔH (J/g)	-20	9.82±0.03[a]	8.69±0.04[b]	3.77±0.01[c]	7.15±0.02[d]	8.65±0.02[b]
	-50		9.45±0.01[b]	8.99±0.01[c]	9.38±0.02[d]	9.42±0.03[d]

		-80	10.46±0.02[b]	9.75±0.03[c]	10.19±0.02[d]	10.33±0.02[e]

T_o, T_p, T_c, ΔH indicate the gelatinization parameter onset, peak, and conclusion temperatures and the gelatinization enthalpy, respectively.
Mean of three measurements ± standard deviation.
Values for the same processing conditions and different additive groups with different letters are significantly different (p<0.05).

Table 4 Pasting properties of native wheat starch and starch-oligosaccharides at different freezing temperatures. (NWS: native wheat starch; FWS: freezing-treated wheat starch; FOS: freezing-treated wheat starch with fructooligosaccharides; XOS: freezing-treated wheat starch with xylooligosaccharides; GOS: freezing-treated wheat starch with galactooligosaccharides.)

	Index	Temperature	NWS	FWS	FWS-XOS	FWS-GOS	FWS-FOS
RVA	Pasting temperature (°C)	-20	92.95±0.18[a]	91.35±0.23[b]	92.15±0.34[c]	92.95±0.18[a]	93.15±0.28[a]
		-50		91.35±0.31[b]	92.35±0.24[c]	93.35±0.23[d]	94.55±0.28[e]
		-80		92.05±0.22[b]	93.75±0.27[c]	93.75±0.32[d]	94.50±0.24[e]
	Peak viscosity (cP)	-20	1499.52±5.93[a]	1828.77±2.85[b]	1631.11±4.02[c]	1558.39±3.54[d]	1495.91±2.53[a]
		-50		1601.71±4.82[b]	1512.39±3.54[c]	1514.46±2.51[c]	1448.83±5.37[d]
		-80		1599.24±5.56[b]	1440.44±3.36[c]	1488.82±5.05[d]	1434.21±1.92[e]
	Breakdown	-20	517.36±1.62[a]	357.15±1.25[b]	301.48±0.97[c]	282.55±0.94[d]	294.28±0.27[e]
		-50		403.66±1.21[b]	367.73±0.85[c]	338.44±1.24[d]	319.02±1.16[e]

viscosity (cP)	-80		454.19±0.87[b]	383.11±1.14[c]	410.85±1.08[d]	414.63±0.89[e]
Setback viscosity (cP)	-20	901.27±2.38[a]	687.73±2.15[b]	642.17±2.29[c]	657.74±1.81[d]	674.64±1.86[e]
	-50		745.41±3.14[b]	653.64±1.32[c]	686.39±0.94[d]	699.24±1.38[e]
	-80		919.67±3.49[b]	821.42±1.03[c]	829.12±3.04[d]	858.33±1.53[e]

Mean of three measurements ± standard deviation.
Values for the same processing conditions and different additive groups with different letters are significantly different (p<0.05).

- Freezing promotes double-helix disruption and retrogradation of starch granules.
- Higher freezing temperatures result in more damage by ice crystals.
- Oligosaccharides reduce frozen ice crystal damage and starch retrogradation.
- A 16% xylooligosaccharides content has a good antifreeze effect.
- The antifreeze effect of oligosaccharides is related to their structure.